



Chemical composition and antifungal effects of three species of *Satureja* (*S. hortensis*, *S. spicigera*, and *S. khuzistanica*) essential oils on the main pathogens of strawberry fruit

Mohsen Farzaneh^a, Hossein Kiani^{b,*}, Rohollah Sharifi^c, Mojtaba Reisi^d, Javad Hadian^a

^a Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C. Evin, Tehran, Iran

^b Bioprocessing and Biodetection Lab, Department of Food Science and Technology, University of Tehran, Karaj, Iran

^c Department of Plant Protection, Razi University of Kermanshah, Kermanshah, Iran

^d Department of Public Health, Cereal Health research Centre, School of Health, Golestan University of Medical Science, Gorgan, Iran

ARTICLE INFO

Article history:

Received 19 March 2015

Received in revised form 21 June 2015

Accepted 29 June 2015

Available online 13 July 2015

Keywords:

Antifungal activity

Essential oil

Satureja

Strawberry

Biological control

ABSTRACT

Due to an increasing risk of chemical contamination upon the application of synthetic fungicides to preserve fresh fruits and vegetables, essential oils are gaining increasing attentions. In this research, besides chemical analysis of the essential oils of three *Satureja* species (*S. hortensis*, *S. spicigera*, and *S. khuzistanica*) by GC–MS, their fungicidal and/or fungistatic effects on postharvest pathogens of strawberry were investigated. Essential oils were extracted by means of hydro-distillation and afterwards GC/MS analysis was performed to identify their components. Carvacrol, γ -terpinene and *p*-cymene were detected as the repeating main constituents of the spices, while thymol and carvacrol methyl ether were found as major components only in *S. spicigera* oil. *In vitro* results showed that at the maximum concentration, the essential oils did not possess fungicidal effects on *Aspergillus niger* but they exhibited fungicidal activities against *Penicillium digitatum*, *Botrytis cinerea* and *Rhizopus stolonifer*. However, *S. khuzistanica* was the strongest oil in fungicidal activity. *S. hortensis* oil was more effective than *S. spicigera* against *B. cinerea* whereas *S. spicigera* oil showed stronger fungicidal activity against *R. stolonifer*. In conclusion, essential oils isolated from three savory species could be suitable for applications in the food industry to control molds and improve the safety of fruits and vegetables.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Basic production resources have been a subject of risk due to the increasing amount of environmental pollutants and extravagant utilization of food and agricultural products. Application of agrochemical products and other contaminants is a serious challenge of the current century which has resulted in destruction of numerous vast lands and reduction of the quality of water resources. Based on this fact, the idea of sustainable production and improved safety of the basic resources have been developed all around the world, especially in the section of agricultural products that organic foods and natural based treating agents have been subjects of research and advancement (Adeyinka and Richard, 2015; Juneja et al., 2012; Prakash et al., 2012).

Fungi are the main deteriorating means of foodstuffs such as fruits and cereals during storage period that are able to diminish

the quality attributes and nutritional values by their growth or by production of toxic compounds (Cardenas-ortega et al., 2005; Mishra and Dubey, 1994). Storage fungi are commonly controlled by synthetic chemicals; however, these agents usually impact the human health and the environment adversely (Nakahara et al., 2013). Furthermore, the use of fungicides is more harmful in the post-harvest period because of the short time between treatment and consumption. Some fungi have shown resistance against broad spectrum fungicides such as benzimidazoles, imazalil and prochloraz due to repeated usage and some of them such as *Mucor* and *Rhizopus* are not sensitive and need especial fungicides to be controlled (Lima et al., 2015; Mari et al., 2014; Sánchez-Torres and Tuset, 2011; Sharifi et al., 2008). Benzimidazoles, the widest applied fungicides in postharvest period, have no effect on Dematiaceous fungi (dark spore fungi), Oomycetes, *Mucor* and *Rhizopus* (two later are major postharvest pathogens of numerous products). In addition, imazalil and prochloraz are ineffective against *Rhizopus stolonifer* (Agrios, 2005).

A number of plants produce natural antifungal compounds in their tissues defending them against biological hazards by

* Corresponding author. Fax: +98 26 32249453.
E-mail address: hokiani@ut.ac.ir (H. Kiani).

exhibiting non-host resistance against most of potential pathogens (Mysore and Ryu, 2004). Selected plants and their essential oils have been evaluated as natural sources for controlling storage fungi (Cardenas-ortega et al., 2005). Essential oils are volatile materials containing a complex mixture of compounds, mainly monoterpenes, sesquiterpenes and phenylpropanoids (Fujita and Kubo, 2004). Since the chemical composition of the oils is complex, resistance against them is rarely developed (Gómez-Castillo et al., 2013; Siroli et al., 2015) and generally, a combined effect of both active and inactive compounds accounts for the antifungal activity of the oils (Burt, 2004; Guerreiro et al., 2015; Khoury et al., 2014; Sharifi et al., 2008).

Strawberry is a very sensitive fruit against pathogens, and fungal contamination is common in this product due to its cultivation style, its probable direct contact to the soil, and its sensitive and soft tissue. Major threatening fungi that reduce the post-harvest storage life of strawberries include *Botrytis*, *Aspergillus*, *Rhizopus* and *Penicillium* (Lazar et al., 2010; Sharma, 2014). Chemical fungicides are generally effective against these pathogens; however, according to the former discussions, they have harmful effects on the human health and are not acceptable choices.

The application of essential oils and plant extracts for controlling post-harvest pathogens has been a subject of interest with a developing trend. These substances are natural products with no adverse effects on the environment and increase the quality and shelf life of fruits due to their anti-oxidant activity (Daniel et al., 2015; Elshafie et al., 2015; Sivakumar and Bautista-Banos, 2014). Identification of biologically active compounds being potential as bio-controlling natural products for the post-harvest storage would be valuable. Plants belonging to the genus *Lamiaceae* and *Apiaceae* are generally rich of anti-microbial and anti-oxidant ingredients and their extracts or essential oils are potentially advantageous to be used as antifungal products (Anthony et al., 2003; Arras and Usai, 2001; Chen et al., 2014; Mohammadi et al., 2014; Rasooli and Mirmostafa, 2003; Senhaji et al., 2014; Sivakumar and Bautista-Banos, 2014; Spadaro and Gullino, 2014).

The genus *Satureja* (Lamiaceae, subfamily *Nepetoideae* and tribe *Satureja*) constitutes about 200 species of herbs and shrubs, often aromatic, widely distributed in Mediterranean area, Asia and boreal America (Cantino et al., 1992). Evidently, members of the genus *Satureja*, including a well-known species *Satureja hortensis* L., known as summer savory, *Satureja montana* L., or winter savory and *Satureja cuneifolia* Ten. or wild savory are natural sources for different applications. *Satureja*, as an aromatic plant, is frequently used in local spices and is known as a traditional medicinal plant (Čavar et al., 2008; Lawless, 2013; Skočibušić et al., 2006). Some members of this genus are of economic importance since they have been used as culinary herbs, flavouring agents in perfumery and cosmetics. The positive effects of savory on human health have now been attributed to its various biologically active constituents such as essential oil, triterpenes (Escudero et al., 1985) and flavonoids (Tomás-Barberán et al., 1988). The essential oil of savory contains antioxidative compounds, namely carvacrol, thymol, β -caryophyllene, γ -terpinene, *p*-cymene, together with linalool, which has been reported to possess strong antioxidant effects (Ruberto and Baratta, 2000) and also has dipentane, ursolic acid, etheral oil, phenolic substances, resins, tannins and mucilage (Lawrence, 2000). The oil exhibited differences in *p*-cymene, myrcene and γ -terpinene contents. *S. hortensis* L. ('summer savory') is a well-known medicinal plant which is widely distributed in different parts of Iran as one of the most important of twelve classified *Satureja* species. Besides of its usual use in food industry as an aromatic and flavouring agent, it has been received major consideration for having anti-inflammatory (Lawrence, 2000),

antioxidant (Güllüce et al., 2003), antibacterial (Güllüce et al., 2003; Şahin et al., 2003) and antifungal activities (Güllüce et al., 2003; Kotan et al., 2013; Razzaghi-Abyaneh et al., 2008; Rezvanpanah et al., 2011). *S. spicigera*, and *S. khuzistanica* are among other species of *Satureja* found in Iran.

The aim of this study was to analyze and compare the chemical composition of the aerial parts of the three species of *Satureja* growing in Iran (*S. hortensis*, *S. spicigera*, and *S. khuzistanica*), evaluate their potential to be utilized as antifungal agents and examine the effect of these essential oils on the main fungal pathogens of strawberries (as a valuable and vulnerable fruit generally requiring major fungicidal treatments).

2. Materials and methods

2.1. Preparation of essential oils

Aerial parts of fresh *S. hortensis*, *S. spicigera*, and *S. khuzistanica* were collected from Lorestan province, Iran in summer 2008, dried in dark at room temperature and then powdered with a blender after verifying the species and elimination of trash bodies (Sharifi et al., 2008). The essential oils were isolated by hydro-distillation, using a Clevenger-type apparatus and then were dried and stored at 4 °C.

2.2. GC-FID and GC-MS analysis

GC analysis was performed with a Thermoquest (San Jose, CA) gas chromatograph with a flame ionization detector (FID). Analysis was carried out using fused silica capillary DB-1 column (60 m \times 0.25 mm i.d.; film thickness = 0.25 μ m). Injector and detector temperatures were 250 °C and 300 °C, respectively. Helium was used as the carrier gas at a flow rate of 0.018 mLs⁻¹; oven temperature was programmed from 60 °C to 250 °C at the rate of 0.067 °C s⁻¹, and finally held isothermally for 60 s.

GC-MS analysis was also performed by using Thermoquest-Finnigan (San Jose, CA) gas chromatograph equipped with column described above and coupled with a TRACE mass quadrupole detector. Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200 °C and 250 °C, respectively.

2.3. Identification of components

The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes C₆–C₂₄. Identification of individual compounds was carried out by comparison of their mass spectra with those of similar compounds from a database (Wiley/NBS library) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (Özcan et al., 2006). For quantification purpose, relative area percentages obtained by FID, were used without using correction factors.

2.4. Fungal inoculum preparation

Strains of postharvest fungi, *Aspergillus niger*, *Penicillium digitatum*, *Botrytis cinerea*, and *R. stolonifer*, isolated from strawberry store were provided by the Department of Plant Pathology, University of Tehran. Cultures of the microorganisms were maintained on potato dextrose agar (PDA) medium.

Spore suspensions were provided from 10 day old cultures of each fungus on PDA and their population was adjusted to 1×10^5 spore per mL by hemocytometer. For inoculation, mycelium was taken from the periphery of 4-day-old stock cultures.

2.5. Toxicity study

2.5.1. Inhibition of mycelial growth

The toxicity of the essential oils against tested fungi was studied by two methods including poisonous medium technique using potato dextrose agar (PDA) medium and broth micro-dilution method (Mishra and Dubey, 1994). Six concentrations (0, 75, 150, 300, 600 and 1200 $\mu\text{L L}^{-1}$) were mixed with sterile molten PDA (cooled to 40 °C), and a surfactant (Tween 80) was also added (0.05%) to facilitate oil dispersion. For inoculation, plugs of mycelium were taken from the periphery of 4-day-old stock cultures and were inverted and were placed in the center of each Petri dish (9 cm diameter). Four replicate plates were sited up for each concentration and plates were incubated in the dark at 27 °C. According to Cakir et al. (2005), growth inhibition of treated samples (T) against control (C) was calculated by percentage, using the following formula:

$$\% \text{Inhibition} = \frac{C - T}{C} \times 100$$

where C is an average of four replicates of hyphal extension (mm) of controls and T is an average of four replicates of hyphal extension (mm) of plates treated with essential oils. For the detection of fungistatic or fungitoxic effect in the samples with inhibited growth of fungi, fungal discs were re-inoculated onto fresh mediums and revival of fungal growth was recorded in 27 °C after ten days. In the second method, to determine the percent of inhibition, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of essential oils against tested fungal strains broth microdilution method was performed. For this purpose, different concentrations of the essential oils (75, 150, 300, 600 and 1200 $\mu\text{L L}^{-1}$) were prepared in PDB (Potato Dextrose Broth) medium. Meanwhile, suspensions of pathogenic fungi spores containing 10^5 spores per mL were also prepared. 30 μL portions of each fungi suspension were mixed with 150 μL of the essential oil solutions and the obtained cultured media were transferred to 96 well plates. The samples were incubated at 27 °C for 72 h while shaken using an incubator shaker. The lowest concentrations of the essential oils with no fungal growth were read as MIC. Minimum fungicidal concentrations were determined by re-culturing of the samples with no fungal growth in PDB (100 μL media for each 10 μL of the samples) and incubation at 27 °C for 72 h with shaking. The experiments were replicated for four times and a control sample was also prepared using sterile distilled water. For both of the above mentioned methods, a total of 20 treatments (four replications of five concentrations) were carried out for each oil in addition to the control sample (four replications).

2.5.2. Inhibition of spore germination

Suspensions of fungi spores containing 10^5 spores per mL were produced in distilled water. Afterwards, 50 μL of each fungal suspension was mixed with the essential oil solutions to achieve a final concentration of 600 $\mu\text{L L}^{-1}$ of the oil in the final medium volume of 0.2 mL. After incubation of the samples at 27 °C for 8 h, germination of the spores was evaluated using light microscopy and the number of germinated spores among around 100 counted spores was measured. Spores were considered as germinated when the length of the hypha was longer than the half of the radius of the spore (Plascencia-Jatomea et al., 2003; Regnier et al., 2014).

2.6. Data analysis

If needed, Probit analysis was used to measure MIC with SPSS 9. MIC assumed as minimum level of essential oil concentration with 95% reduction of the fungi growth (Eckert and Ogawa, 1988). Were

needed, ANOVA analysis and means comparison test (LSD) were performed using SPSS 9 software.

3. Results and discussion

3.1. Chemical composition of the essential oils

The yield of the isolated essential oils of *S. hortensis*, *S. spicigera* and *S. khuzistanica* were 2.4, 3.7 and 3.9%, respectively. The GC and GC–MS analyses of the essential oils permitted the identification of 29, 24 and 24 principal constituents making a total of 99.9, 98.8 and 98.8% of the oils for *S. hortensis*, *S. khuzistanica* and *S. spicigera*, respectively (Tables 1). Carvacrol (48%), γ -terpinene (24.2%) and *p*-cymene (11.7%) were the main components of *S. hortensis* oil. The main components of *S. khuzistanica* oil were carvacrol (48%), *p*-cymene (18.5%) and γ -terpinene (11%). Thymol (29.5%), *p*-cymene (23.4%), γ -terpinene (15.2%), carvacrol (9.6%) and carvacrol methyl ether (8.5%) were the main components of *S. spicigera* oil. Carvacrol, γ -terpinene, and *p*-cymene were detected as the repeating main components of the three spices while thymol and carvacrol methyl ether were only found in *S. spicigera* among the major components, so that thymol, as the first major compound, consisted up to one out of three of all components in this species. Whereas carvacrol accounted for around half of the components of *S. hortensis*, and *S. khuzistanica* oils, the concentration of this compound was significantly lower in *S. spicigera* oil. The highest amount of γ -terpinene was observed for *S. hortensis* oil followed by *S. khuzistanica* oil. On the other hand, the highest concentration of *p*-cymene was shown to be in *S. spicigera* oil compared to the oils of other two species.

Literature review has shown variations between chemical composition of different *Satureja* species oils

Table 1

Chemical composition (relative area percentage) of the essential oils of the three species of *Satureja* (*S. hortensis*, *S. khuzistanica*, and *S. spicigera*).

No.	Compound	RI	<i>S. hortensis</i>	<i>S. khuzistanica</i>	<i>S. spicigera</i>
1	α -thujene	925	2.3	1.2	0.1
2	α -pinene	933	2.5	1.6	0.4
3	Camphene	947	0.2	0.1	–
4	1-octene-3-ol	960	0.1	–	–
5	β -pinene	974	1.6	1.2	1.4
6	Myrcene	981	2.5	1.7	1
7	α -phelandrene	999	0.5	0.2	–
8	δ -3-carene	1007	0.1	–	–
9	<i>p</i> -cymene	1014	11.7	11.0	23.4
10	1,8-cineole	1023	1.0	0.7	0.6
11	(<i>z</i>)- β -ocimene	1036	0.2	–	–
12	γ -terpinene	1053	24.2	18.5	15.2
13	<i>cis</i> -sabinene hydrate	1056	0.2	0.2	0.1
14	α -terpinene	1080	0.1	–	–
15	Linalool	1085	0.1	0.2	3.8
16	<i>trans</i> -sabinene hydrate	1055	0.5	0.2	0.2
17	<i>trans</i> -2-carene-4-ol	1145	–	0.1	0.1
18	4,5-epoxy-carane	1151	0.1	–	0.1
19	terpin-4-ol	1163	0.5	0.5	2.3
20	α -terpineol	1175	0.2	0.2	–
21	thymol methyl ether	1225	0.1	–	0.2
22	carvacrol methyl ether	1237	–	–	8.1
23	Thymol	1266	0.3	0.3	29.5
24	Carvacrol	1282	48.0	57.4	9.6
26	Carvacryl acetate	1345	1.0	2.1	1.4
27	β -caryophyllene	1424	0.9	0.4	0.4
29	β -bisabolene	1501	1.0	0.7	0.5
30	Spathulenol	1576	0.2	0.1	0.2
31	caryophyllene oxide	1960	0.1	0.3	0.2
Total			99.9	98.8	98.8

(Sefidkon and Jamzad, 2005). There are many reports on the composition of essential oils of the aerial parts and leaves of savory species from different parts of the world (Góra et al., 1996; Hajhashemi et al., 2002; Hajhashemi et al., 2000). Tozlu et al. (2011) and Hadian et al. (2010) reported carvacrol, γ -terpinene, *p*-cymene, and α -terpinene as the main components of *S. hortensis* essential oil. Sefidkon et al. (2006) identified twenty three components in the *S. hortensis* oil including carvacrol and γ -terpinene as the main components. Farsam et al. (2004) determined the main components of the *S. khuzistanica* oil as carvacrol, *p*-cymene and Thymol. Eminagaoglu et al. (2007) reported carvacrol, γ -terpinene, and *p*-cymene as the major constituents of *S. spicigera* essential oil.

3.2. Antifungal activity of the essential oils on PDA cultures

The results of inhibition indices percentage of different concentration of essential oils are presented in Fig. 1. As can be observed in this figure, there was no significant inhibitory activity against fungi at concentration of 75 μL oil per L PDA. However, increasing the concentration of this oil led to a decreasing trend in the growth of all tested fungi strains. *S. khuzistanica*, in concentrations equal to 600 $\mu\text{L L}^{-1}$ or higher, completely inhibited the growth of all tested fungi strains. Inhibitory effect of *S. hortensis* and *S. spicigera* manifested a similar pattern: 75 and 150 $\mu\text{L L}^{-1}$ of these oils lowered the growth potential of all studied fungi strains and concentrations higher than 300 $\mu\text{L L}^{-1}$ could completely inhibit the growth of the tested strains.

All of the savory species exhibited considerable antifungal activity against *P. digitatum*, *B. cinerea* and *R. stolonifer* with MICs of 600 $\mu\text{L L}^{-1}$, 300 $\mu\text{L L}^{-1}$ and 300 $\mu\text{L L}^{-1}$, respectively (Table 2). Results from the re-inoculation of the examined samples to a fresh medium for indication of fungicidal and/or fungistatic effect of the oils showed that at the maximum concentration used (1200 $\mu\text{L L}^{-1}$ of the medium) none of the savory species possessed fungicidal effects on *A. niger* but *S. hortensis* and *S. spicigera* had fungicidal effects against *P. digitatum*. Furthermore, *S. khuzistanica* exhibited fungicidal activity on *B. cinerea* and *R. stolonifer*. The minimum fungicidal concentrations of the essential oil of *S. hortensis* on *B. cinerea* and *R. stolonifer* were 300 and 600 $\mu\text{L L}^{-1}$, respectively and *S. spicigera* showed fungicidal activity on *B. cinerea* and *R. stolonifer* at the concentrations of 1200 and 300 $\mu\text{L L}^{-1}$, respectively (Table 2).

A similar observation (Fig. 2) with RI% determination by poisonous medium technique was made using broth micro-dilution. The RI% of essential oils against fungal strains was ranged 0–100% which is approximately lower than those of poisonous medium technique. In general, it can be concluded that the essential oils exhibited stronger antimicrobial activity against fungal strains using broth micro-dilution method.

Table 3 shows the MIC and MFC ranges of the essential oils obtained by broth microdilution for different fungal groups. MIC and MFC determined ranged from 600 to 1200 $\mu\text{L L}^{-1}$, whereas the MICs and MFCs obtained by poisonous medium technique were in range of 300–1200 $\mu\text{L L}^{-1}$. In general MICs assessed by broth microdilution were almost always one to several times lower than those generated by poisonous medium technique.

Results obtained after determining the germination of spores are presented in Table 4. The number of germinated spores of *A. niger*, *P. digitatum*, *B. cinerea*, *R. stolonifer* were significantly lower than those of control. For instance, *S. khuzestanica* oil completely inhibited the growth of *B. cinerea* and *R. stolonifer* and *A. niger* and *P. digitatum* grew in low numbers. *S. hortensis* and *S. spicigera* performed in a similar way. All of the examined essential oils showed comparable patterns to prevent the growth of fungal strains.

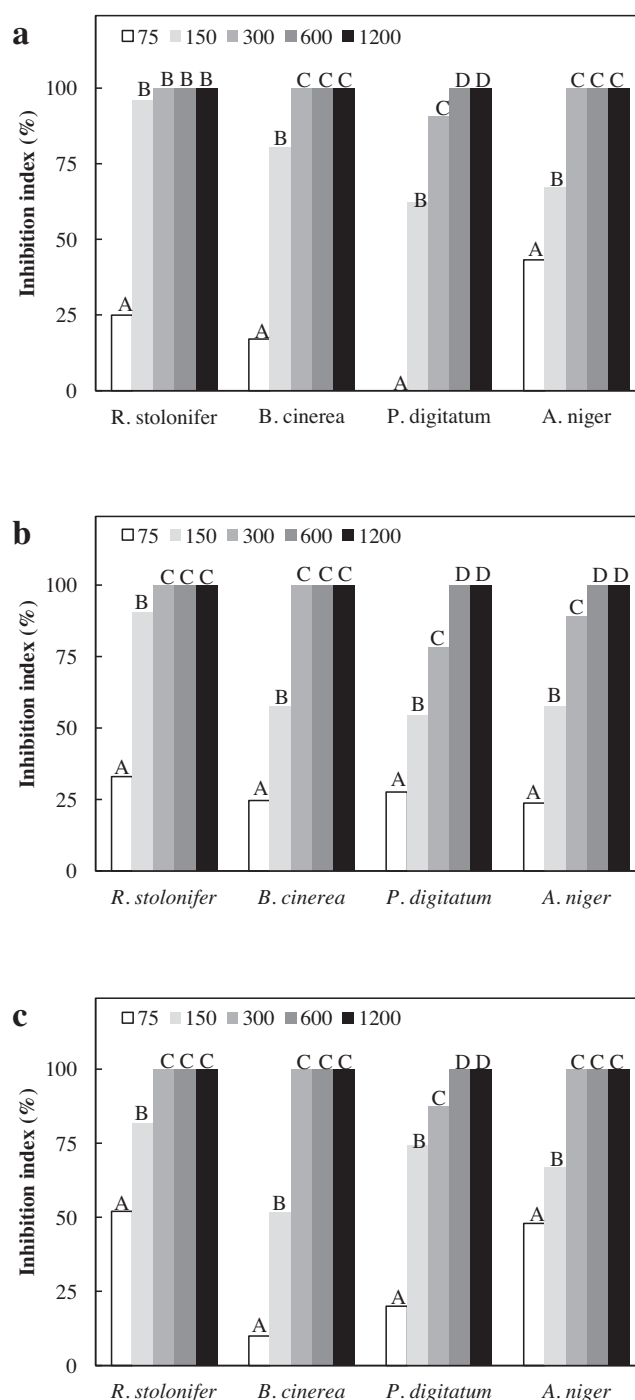


Fig. 1. Inhibition indices percentage of various concentrations of *S. khuzistanica* (a), *S. hortensis* (b) and *S. spicigera* (c) on the growth inhibition of fungi strains causing strawberry rot (columns for each fungus strain from left to right denote 75, 150, 300, 600, and 1200 $\mu\text{L L}^{-1}$). The experiments were carried out *in vitro* by poisonous medium technique using potato dextrose agar. Different letters for each fungal strain indicate significant differences among treatments ($P \leq 0.05$).

The results of this study showed that the evaluated essential oils could exhibit potent inhibitory effects against *A. niger*, *P. digitatum*, *B. cinerea* and *R. stolonifer*, the main deteriorating fungi strains found on strawberries. The antifungal effect of savory species could be due to the high contents of phenolic compounds such as major monoterpene constituents (Thymol and carvacrol). It has been reported that thymol and carvacrol are

Table 2

Minimum inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the essential oils of savory species against fungal strains causing strawberry rot. The experiments were carried out *in vitro* by poisonous medium technique using potato dextrose agar medium ($\mu\text{L L}^{-1}$).

	Fungi	Savory species		
		<i>S. khuseztanica</i>	<i>S. hortensis</i>	<i>S. spicigera</i>
MIC ($\mu\text{L L}^{-1}$)	<i>A. niger</i>	300 ^{A*}	600 ^A	300 ^A
	<i>P. digitatum</i>	600 ^B	600 ^A	600 ^B
	<i>B. cinerea</i>	300 ^A	300 ^B	300 ^A
	<i>R. stolonifer</i>	300 ^A	300 ^B	300 ^A
MFC ($\mu\text{L L}^{-1}$)	<i>A. niger</i>	>1200	>1200	>1200
	<i>P. digitatum</i>	1200 ^A	1200 ^A	>1200
	<i>B. cinerea</i>	1200 ^A	600 ^B	1200 ^A
	<i>R. stolonifer</i>	1200 ^A	300 ^C	300 ^B

* Different superscripts in each column for each essential oil indicate significant differences among treatments ($P \leq 0.05$).

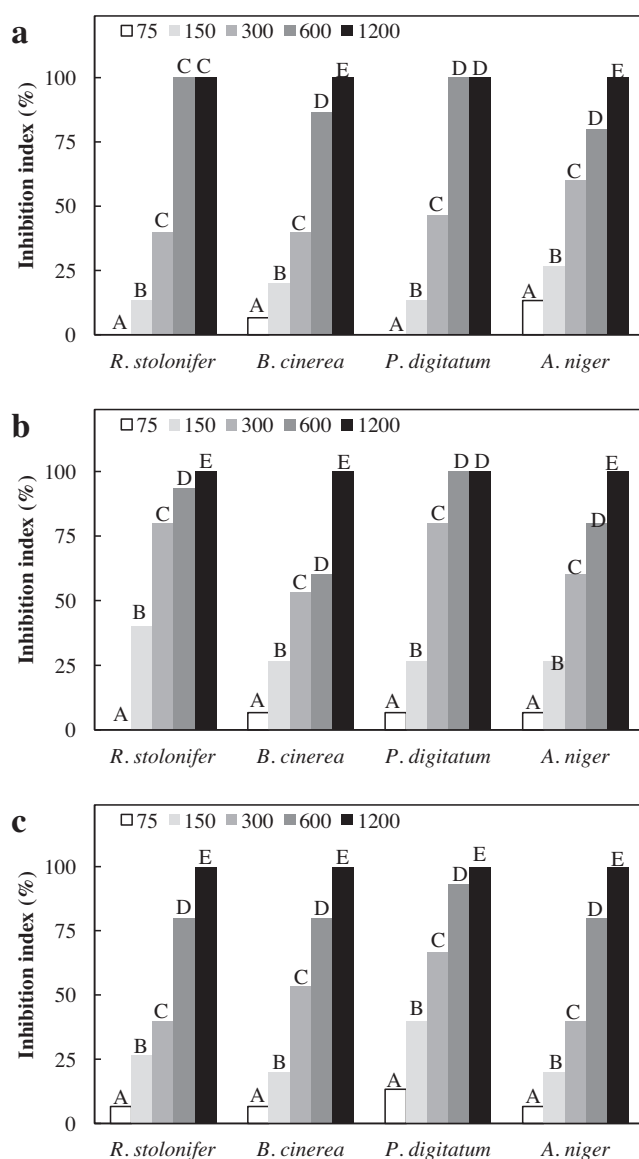


Fig. 2. Inhibition indices percentage of various concentrations of *S. khuzistanica* (a), *S. hortensis* (b) and *S. spicigera* (c) on the growth inhibition of fungi strains causing strawberry rot (columns for each fungus strain from left to right denote 75, 150, 300, 600, and 1200 $\mu\text{L L}^{-1}$). The experiments were carried out *in vitro* by broth microdilution method. Different letters for each fungal strain indicate significant differences among treatments ($P \leq 0.05$).

more effective on bacterial and fungal strains than γ -terpinene and *p*-cymene and have stronger antifungal properties (Kordali et al., 2008). Metabolic pathways for the thymol and carvacrol formation starts with the auto-oxidation of γ -terpinene to *p*-cymene (Brewer, 2011). The subsequent hydroxylation to thymol and unsaturation of γ -terpinene to *p*-cymene after hydroxylation to C-2 aromatic ring is the primary mechanism of carvacrol formation (Brewer, 2011). Thus, it can be assumed that γ -terpinene and *p*-cymene played key roles in the process of flavoring and as precursor of oxygenated compounds. It should also be mentioned that the mod of action of essential oils in not limited to a single compound and is usually derived from the interactions and synergistic effects of different constituents present in the oil (Sharifi et al., 2008). Researches has revealed that the essential oils isolated from savory and other similar plants could exhibit antifungal effect (Conner and Beuchat, 1984; Omidbeygi et al., 2007). The main antifungal activity of other similar essential oils containing high amounts of phenolic compounds has been reported as creating severe lesions of the membrane due to direct and metabolic impairment leading to secondary membrane damage. Other mechanisms include affecting the functional integrity of mitochondria, inhibiting the synthesis of ATP in the mitochondria of fungi strains and accumulation of ROS after using essential oils that this phenomenon is considered as one of the primary biochemical hall marks of apoptosis promoting morphological changes, nuclear fragmentation, chromatin condensation, cellular swelling and phosphatidylserine externalization (Kubo et al., 2003; Wink and Schimmer, 2010).

Table 3

Minimum inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the essential oils against fungal strains causing strawberry rot. The experiments were carried out *in vitro* by broth microdilution method.

	Fungi	Savory species		
		<i>S. khuseztanica</i>	<i>S. hortensis</i>	<i>S. spicigera</i>
MIC ($\mu\text{L L}^{-1}$)	<i>A. niger</i>	1200 ^{A*}	1200 ^A	1200 ^A
	<i>P. digitatum</i>	600 ^B	600 ^B	1200 ^A
	<i>B. cinerea</i>	600 ^B	1200 ^A	1200 ^A
	<i>R. stolonifer</i>	600 ^B	1200 ^A	1200 ^A
MFC ($\mu\text{L L}^{-1}$)	<i>A. niger</i>	>1200	>1200	>1200
	<i>P. digitatum</i>	>1200	>1200	>1200
	<i>B. cinerea</i>	>1200	>1200	>1200
	<i>R. stolonifer</i>	1200	1200	>1200

* Different superscripts in each column for each essential oil indicate significant differences among treatments ($P \leq 0.05$).

Table 4Germination of spores of different fungi treated by savory essential oils (600 $\mu\text{L L}^{-1}$).

	Fungi	Savory species			
		<i>S. khuzestanica</i>	<i>S. hortensis</i>	<i>S. spicigera</i>	Control
Germination (%)	<i>A. niger</i>	7 ^A	5 ^A	15 ^B	99 ^C
	<i>P. digitatum</i>	3 ^A	0 ^A	7 ^A	100 ^B
	<i>B. cinerea</i>	0 ^A	4 ^A	10 ^B	100 ^C
	<i>R. stolonifer</i>	0 ^A	3 ^A	6 ^A	99 ^B

^a Different superscripts in each row indicate significant differences among treatments ($P \leq 0.05$).

4. Conclusion

According to the results of this study, it is reported that essential oils from savory species could be suitable for applications in the food industry and owing to their natural origin as well as having benefits for the environment the interest of using these essential oils for food and fruits safety and preservation could be increased.

References

- Adeyinka, A., Richard, F., 2015. Application of phytochemical extracts and essential oils in food products: A review. *Int. J. Biotechnol. Food Sci.* 3 (3), 31–35.
- Agrios, G., 2005. *Plant Pathology*, 5th ed. Academic Press, New York.
- Anthony, S., Abeywickrama, K., Wijeratnam, S.W., 2003. The effect of spraying essential oils of *Cymbopogon nardus*, *Cymbopogon flexuosus* and *Ocimum basilicum* on postharvest diseases and storage life of Embul banana. *J. Hortic. Sci. Biotechnol.* 78 (6), 780–785.
- Arras, G., Usai, M., 2001. Fungitoxic activity of 12 essential oils against four postharvest citrus pathogens: chemical analysis of *Thymus capitatus* oil and its effect in subatmospheric pressure conditions. *J. Food Prot.* 64 (7), 1025–1029.
- Brewer, M., 2011. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Compr. Rev. Food Sci. Food Saf.* 10 (4), 221–247.
- Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* 94 (3), 223–253.
- Cakir, A., Kordali, S., Kilic, H., Kaya, E., 2005. Antifungal properties of essential oil and crude extracts of *Hypericum linarioides* Bosse. *Biochem. Syst. Ecol.* 33 (3), 245–256.
- Cantino, P., Harley, R., Wagstaff, S., (1992). *Genera of Labiatae status and classification*.
- Cardenas-ortega, N.C., Zavala-Sanchez, M.A., Aguirre-Rivera, J.R., Perez-Gonzalez, C., Perez-Gutierrez, S., 2005. Chemical composition and antifungal activity of essential oil of *Chrysactinia mexicana* Gray. *J. Agric. Food Chem.* 53, 4347–4349.
- Čavar, S., Maksimović, M., Šolić, M.E., Jerković-Mujkić, A., Bešta, R., 2008. Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. *Food Chem.* 111 (3), 648–653.
- Chen, Q., Xu, S., Wu, T., Guo, J., Sha, S., Zheng, X., Yu, T., 2014. Effect of citronella essential oil on the inhibition of postharvest *Alternaria alternata* in cherry tomato. *J. Sci. Food Agric.* 94 (12), 2441–2447.
- Conner, D., Beuchat, L., 1984. Effects of essential oils from plants on growth of food spoilage yeasts. *J. Food Sci.* 49 (2), 429–434.
- Daniel, C.K., Lennox, C.L., Vries, F.A., 2015. In vivo application of garlic extracts in combination with clove oil to prevent postharvest decay caused by *Botrytis cinerea*, *Penicillium expansum* and *Neofabraea alba* on apples. *Postharvest Biol. Technol.* 99, 88–92.
- Eckert, J.W., Ogawa, J.M., 1988. The chemical control of postharvest diseases: deciduous fruits, berries, vegetables and root/tuber crops. *Annu. Rev. Phytopathol.* 26 (1), 433–469.
- Elshafie, H.S., Mancini, E., Camele, I., De Martino, L., De Feo, V., 2015. In vivo antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit. *Ind. Crops Prod.* 66, 11–15.
- Eminagaoglu, O., Tepe, B., Yumrutas, O., Akpulat, H.A., Daferera, D., Polissiou, M., Sokmen, A., 2007. The in vitro antioxidant properties of the essential oils and methanol extracts of *Satureja spicigera* (K. Koch.) Boiss. and *Satureja cuneifolia* Ten. *Food Chem.* 100 (1), 339–343.
- Escudero, J., Lopez, J.C., Rabanal, R.M., Valverde, S., 1985. Secondary metabolites from *Satureja* species. New triterpenoid from *Satureja acinos*. *J. Nat. Prod.* 48 (1), 128–131.
- Farsam, H., Amanlou, M., Radpour, M., Salehinia, A., Shafiee, A., 2004. Composition of the essential oils of wild and cultivated *Satureja khuzistanica* Jamzad from Iran. *Flavour Fragrance J.* 19 (4), 308–310.
- Fujita, K.-I., Kubo, I., 2004. Potentiation of fungicidal activities of trans-anethole against *Saccharomyces cerevisiae* under hypoxic conditions. *J. Biosci. Bioeng.* 98 (6), 490–492.
- Gómez-Castillo, D., Cruz, E., Iguaz, A., Arroqui, C., Virseda, P., 2013. Effects of essential oils on sprout suppression and quality of potato cultivars. *Postharvest Biol. Technol.* 82 (0), 15–21.
- Góra, J., Lis, A., Lewandowski, A., 1996. Chemical composition of the essential oil of cultivated summer savory (*Satureja hortensis* L. cv. *Saturn*). *J. Essent. Oil Res.* 8 (4), 427–428.
- Guerreiro, A.C., Gago, C.M.L., Faleiro, M.L., Miguel, M.G.C., Antunes, M.D.C., 2015. The effect of alginate-based edible coatings enriched with essential oils constituents on *Arbutus unedo* L. fresh fruit storage. *Postharvest Biol. Technol.* 100 (0), 226–233.
- Güllüce, M., Sökmen, M., Daferera, D., Agar, G., Özkan, H., Kartal, N., Polissiou, M., Sökmen, A., Sahin, F., 2003. In vitro antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. *J. Agric. Food Chem.* 51 (14), 3958–3965.
- Hadian, J., Ebrahimi, S.N., Salehi, P., 2010. Variability of morphological and phytochemical characteristics among *Satureja hortensis* L. accessions of Iran. *Ind. Crops Prod.* 32 (1), 62–69.
- Hajhashemi, V., Ghannadi, A., Pezeshkian, S.K., 2002. Antinociceptive and anti-inflammatory effects of *Satureja hortensis* L. extracts and essential oil. *J. Ethnopharmacol.* 82 (2), 83–87.
- Hajhashemi, V., Sadraei, H., Ghannadi, A.R., Mohseni, M., 2000. Antispasmodic and anti-diarrhoeal effect of *Satureja hortensis* L. essential oil. *J. Ethnopharmacol.* 71 (1), 187–192.
- Juneja, V.K., Dwivedi, H.P., Yan, X., 2012. Novel natural food antimicrobials. *Annu. Rev. Food Sci. Technol.* 3, 381–403.
- Khoury, M., El Beyrouthy, M., Ouaini, N., Iriti, M., Eparvier, V., Stien, D., 2014. Chemical composition and antimicrobial activity of the essential oil of *Juniperus excelsa* M. Bieb. Growing Wild in Lebanon. *Chem. Biodivers.* 11 (5), 825–830.
- Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., Mete, E., 2008. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. *Bioresour. Technol.* 99 (18), 8788–8795.
- Kotan, R., Dadasoglu, F., Karagoz, K., Cakir, A., Ozer, H., Kordali, S., Cakmakci, R., Dikbas, N., 2013. Antibacterial activity of the essential oil and extracts of *Satureja hortensis* against plant pathogenic bacteria and their potential use as seed disinfectants. *Sci. Hortic.* 153, 34–41.
- Kubo, I., Fujita, K.-I., Kubo, A., Nihei, K.-I., Lunde, C.S., 2003. Modes of antifungal action of (2 E)-alkenals against *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* 51 (14), 3951–3957.
- Lawless, J., 2013. *The Encyclopedia of Essential Oils: The Complete Guide to the Use of Aromatic Oils In Aromatherapy, Herbalism, Health, and Well Being*. Conari Press.
- Lawrence, B.M., 2000. Progress in essential oils. *Perfumer & Flavorist* 25 (4), 55–70.
- Lazar, E.E., Jobling, J.J., Benkeblia, N., 2010. Postharvest disease management of horticultural produce using essential oils: today's prospects. *Stewart Postharvest Rev.* 6 (3), 1–9.
- Lima, G., Sanzani, S.M., De Curtis, F., Ippolito, A., 2015. Biological control of postharvest diseases. *Adv. Postharvest Fruit Veg. Technol.* 65.
- Mari, M., Di Francesco, A., Bertolini, P., 2014. Control of fruit postharvest diseases: old issues and innovative approaches. *Stewart Postharvest Rev.* 10 (1), 1–4.
- Mishra, A., Dubey, N., 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Appl. Environ. Microbiol.* 60 (4), 1101–1105.
- Mohammadi, A., Nazari, H., Imani, S., Amrollahi, H., 2014. Antifungal activities and chemical composition of some medicinal plants. *J. Med. Mycol.* 24 (2), e1–e8. *Journal de Mycologie Médicale*.
- Mysore, K.S., Ryu, C.-M., 2004. Nonhost resistance: how much do we know. *Trends Plant Sci.* 9 (2), 97–104.
- Nakahara, K., Alzoreky, N.S., Yoshihashi, T., Nguyen, H.T., Trakoontivakorn, G., 2013. Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus* (citronella grass). *Jpn. Agric. Res. Q.* 37 (4), 249–252.
- Omidbeygi, M., Barzegar, M., Hamidi, Z., Naghdibadi, H., 2007. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. *Food Control* 18 (12), 1518–1523.
- Özcan, M.M., Chalchat, J.-C., Arslan, D., Ates, A., Ünver, A., 2006. Comparative essential oil composition and antifungal effect of bitter fennel (*Foeniculum vulgare* ssp. *piperitum*) fruit oils obtained during different vegetation. *J. Med. Food* 9 (4), 552–561.
- Plascencia-Jatomea, M., Viniegra, G., Olayo, R., Castillo-Ortega, M.M., Shirai, K., 2003. Effect of chitosan and temperature on spore germination of *Aspergillus niger*. *Macromol. Biosci.* 3 (10), 582–586.
- Prakash, B., Singh, P., Kedia, A., Dubey, N., 2012. Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant activities and in vivo efficacy in food system. *Food Res. Int.* 49 (1), 201–208.
- Rasooli, I., Mirmostafa, S.A., 2003. Bacterial susceptibility to and chemical composition of essential oils from *Thymus kotschyianus* and *Thymus persicus*. *J. Agric. Food Chem.* 51 (8), 2200–2205.

- Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., Yoshinari, T., Rezaee, M.-B., Jaimand, K., Nagasawa, H., Sakuda, S., 2008. Inhibitory effects of *Satureja hortensis* L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*. *Int. J. Food Microbiol.* 123 (3), 228–233.
- Regnier, T., Combrinck, S., Veldman, W., Du Plooy, W., 2014. Application of essential oils as multi-target fungicides for the control of *Geotrichum citri-aurantii* and other postharvest pathogens of citrus. *Ind. Crops Prod.* 61, 151–159.
- Rezvanpanah, S., Rezaei, K., Golmakani, M.-T., Razavi, S.H., 2011. Antibacterial properties and chemical characterization of the essential oils from summer savory extracted by microwave-assisted hydrodistillation. *Braz. J. Microbiol.* 42 (4), 1453–1462.
- Ruberto, G., Baratta, M.T., 2000. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* 69 (2), 167–174.
- Şahin, F., Karaman, I., Güllüce, M., Ögütçü, H., Şengül, M., Adıgüzel, A., Öztürk, S., Kotan, R., 2003. Evaluation of antimicrobial activities of *Satureja hortensis* L. *J. Ethnopharmacol.* 87 (1), 61–65.
- Sánchez-Torres, P., Tuset, J.J., 2011. Molecular insights into fungicide resistance in sensitive and resistant *Penicillium digitatum* strains infecting citrus. *Postharvest Biol. Technol.* 59 (2), 159–165.
- Sefidkon, F., Abbasi, K., Khaniki, G.B., 2006. Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis*. *Food Chem.* 99 (1), 19–23.
- Sefidkon, F., Jamzad, Z., 2005. Chemical composition of the essential oil of three Iranian *Satureja* species (*S. mutica*, *S. macrantha* and *S. intermedia*). *Food Chem.* 91 (1), 1–4.
- Senhaji, B., Chebli, B., Ferji, Z., 2014. Antifungal activity of medicinal plants extracts against *Botrytis cinerea* the causal agent of gray mold on tomato. *J. Biol. Agric. Healthcare* 4 (26), 141–147.
- Sharifi, R., Kiani, H., Farzaneh, M., Ahmadzadeh, M., 2008. Chemical composition of essential oils of Iranian *Pimpinella anisum* L. and *Foeniculum vulgare* Miller and their antifungal activity against postharvest pathogens. *J. Essent. Oil Bear. Plants* 11 (5), 514–522.
- Sharma, N., 2014. *Biological Controls for Preventing Food Deterioration: Strategies for Pre-and Postharvest Management*. John Wiley & Sons.
- Siroli, L., Patrignani, F., Serrazanetti, D.I., Tappi, S., Rocculi, P., Gardini, F., Lanciotti, R., 2015. Natural antimicrobials to prolong the shelf-life of minimally processed lamb's lettuce. *Postharvest Biol. Technol.* 103 (0), 35–44.
- Sivakumar, D., Bautista-Banos, S., 2014. A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Prot.* 64, 27–37.
- Skočibušić, M., Bezić, N., Dunkić, V., 2006. Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis. growing in Croatia. *Food Chem.* 96 (1), 20–28.
- Spadaro, D., Gullino, M.L., 2014. Use of essential oils to control postharvest rots on pome and stone fruit. *Post-Harvest Pathology*. Springer, pp. 101–110.
- Tomás-Barberán, F.A., Husain, S.Z., Gil, M.I., 1988. The distribution of methylated flavones in the *Lamiaceae*. *Biochem. Syst. Ecol.* 16 (1), 43–46.
- Tozlu, E., Cakir, A., Kordali, S., Tozlu, G., Ozer, H., Akcin, T.A., 2011. Chemical compositions and insecticidal effects of essential oils isolated from *Achillea gypsicola*, *Satureja hortensis*, *Origanum acutidens* and *Hypericum scabrum* against broadbean weevil (*Bruchus dentipes*). *Sci. Hortic.* 130 (1), 9–17.
- Wink, M., Schimmer, O., 2010. Molecular Modes of Action of Defensive Secondary Metabolites, *Annual Plant Reviews. Functions and Biotechnology of Plant Secondary Metabolites*, 39. Wiley-Blackwell, pp. 21–161.